Molecular aspects of bladder cancer  
Aspectos moleculares do câncer de bexiga

Gelbert Luiz Chamon do Carmo Amorim¹, Denny Fabricio Magalhães Veloso¹, José Carlos Vieira¹, Paulo Roberto Alves¹

ABSTRACT
One of the most important objectives of genetic markers of cancer will be the possible identification of individuals at greatest risk in order to allow better management and prognosis. Many urological tumors were associated to various types of gene alterations with a great number of genes involved in the process, hindering gene therapy. This treatment uses specific techniques and one or several genes are manipulated in the laboratory in order to induce molecular alterations that may block the oncogenic process. The article addresses these issues emphasizing the importance of the new molecular biology techniques.

Keywords: Urinary bladder neoplasms/genetics; Urinary bladder/pathology

RESUMO
Um dos mais importantes objetivos dos marcadores genéticos para o câncer será a possível identificação do indivíduo com maior risco de doença, permitindo melhor conduta e prognóstico. Nos casos dos tumores urológicos, vários tumores têm sido relacionados a vários tipos de alterações genéticas, com um grande número de genes envolvidos, que dificulta muito a terapia gênica que é o processo no qual, por meio de técnicas específicas, gene ou genes são manipulados em laboratório para provocar alterações moleculares capazes de bloquear o processo oncogênico. O presente artigo aborda essas questões enfatizando a importância do advento das novas técnicas de biologia molecular.

Descritores: Neoplasias da bexiga urinária/genética; Bexiga urinária/patologia

INTRODUCTION
The importance of genetics greatly increased in the past years and its study has become necessary to understand several diseases(1). Biological processes have some genetic influence; investigating their determining molecular mechanisms is the object of numerous approaches, possibly due to the development of technologies allowing the study of DNA.

Despite the progress of surgical techniques, effective treatment still requires understanding the phenomena that determine cancer.

Genetic and environmental factors are causes of genome lesions leading to cancer, a genetic disease at somatic level resulting from sequential mutations in genes responsible for cell proliferation, death and differentiation, resulting in genomic instability.

Therefore, the cell where the cascade of mutations was initiated starts multiplying, evolving in sublineages with varied grades of abnormalities and malignancy and generating tumor heterogeneity.

Some of the mechanisms affecting the normal sequence of cell cycle involve mutations in two groups of genes, the oncogenes that are related to the induction of cell division and the tumor suppressor genes that act in oncogenes in proliferation control.

Mutations in suppressor genes can lead to malignant transformation of cells when the allele function is lost, thus causing uncontrolled cell division, abnormal differentiation and deficient apoptosis.

The gene most frequently mutated in different types of cancer in humans, including bladder tumors, is the protein p53(2).

Chromosome instability must be considered in tumor cells, suggesting that cancer could be the result of an imbalance in number of chromosomes, therefore, in the amount of functional genetic information in the cell.

There is still a marked variation in susceptibility to carcinogens among individuals; likewise, several genetically determined factors seem to be related to the risk of cancer, without a correlation with carcinogens. Exogenous factors, such as lifestyle, can determine the

Study carried out at Santa Casa de Belo Horizonte (MG), Brazil.

¹ Department of Urology, Santa Casa de Belo Horizonte (MG), Brazil.

Correspondence to: Gelbert Luiz Chamon do Carmo Amorim – Rua: Manaus, 645 – Santa Efigênia – CEP: 30150-350 – Belo Horizonte – Minas Gerais (MG), Brazil. Tel.: (31) 3774 7500 – e-mail: gelbertchamon@hotmail.com

Received: Jan 13, 2010 – Accepted: Dec 20, 2010
intake of vitamins, antioxidants and compounds that modulate the risk of developing neoplasms.

One of the most important objectives of genetic markers of cancer will be possible identification of individuals at higher risk of developing the disease, allowing better management and prognosis.

Several urological tumors were associated to various types of genetic abnormalities, with a large number of genes involved, which hinders gene therapy.

Gene therapy is the process in which one or more genes are manipulated in a laboratory by specific techniques to cause molecular changes that are able to block the oncogenic process.

BLADDER CANCER

Bladder cancer, especially transitional cell carcinoma (TCC), is the fourth most common tumor in males, in the United States. This cancer has a variable clinical course and prognosis, and 70% presenting as a superficial tumor upon diagnosis. However, over 60% of patients have recurrence after endoscopic resection, and a major concern is that in 30% of them it will occur in a more advance grade and/or stage, showing disease progression. About 5% to 10% of patients with initially superficial tumors will develop invasive disease with involvement of bladder muscles

Among patients with radically treated local invasive disease, 50% will develop metastatic disease. Several prognostic markers have been studied to predict which superficial tumors would become invasive and which invasive tumors tend to produce metastases.

Gene **p53**

Upon development of DNA analysis, by means of cloning techniques, a large number of genes were found to be involved in the onset and progression of several types of cancer.

Special importance has been given to mutations in the suppressor gene **p53**, considered to be the most studied genetic defect in bladder cancer

This gene, located in the short arm of chromosome 17, seems to act in encoding a nuclear protein of 53kDa, which has an effect in transcriptional regulation of cell cycle. Mutations of **p53** cause increased expression and half-life of this non-functioning protein, with consequent loss of its suppressing activity, so that this is now detected by immunohistochemistry.

Detection of nuclear accumulation of protein **p53** by immunohistochemistry is strongly associated to **p53** mutations; however, the absence of immunoreactivity does not exclude this genetic abnormality. Reactivity of this protein was demonstrated in 50% of patients with bladder cancer (TCC), specially in high grade and advanced stage tumors. Higher occurrence of mutations in **p53** was also demonstrated in invasive tumors when compared to superficial ones. Fujimoto et al. found mutant **p53** in 50% of patients with invasive tumors and only 7.6% in superficial tumors.

Several authors studied **p53** abnormalities and observed a direct relation between the occurrence of genetic abnormalities and the chances of disease progression and death.

Observation of mutations in superficial tumors was reported in 22% to 64% of patients, with a direct relation between likelihood of recurrence and decreased survival. Hudson et al. demonstrated 71% of progression to invasive disease in **p53** positive patients compared to 22% in patients with undetectable protein.

Another important factor is decreased survival related to disease in patients treated by radical cystectomy due to localized disease who present increased **p53** expression, demonstrating it is an important indicator, regardless of the grade and stage of the disease.

It is known that superficial recurrences usually occur with diploid tumors and negative **p53** mutants and tumors with metastases are aneuploid and **p53**-positive, but both have no prognostic advantages considering cell proliferation rates.

In regard to carcinoma in situ, a strong associated was observed between increased expression of **p53** expression and chances of disease progression and decreased disease-specific survival. In this analysis, 48% showed increased **p53** expression and 86.7% of them progressed.

More recently, some authors demonstrated that **p53** positivity can be converted after immunotherapy with BCG; therefore, it was also demonstrated that the likelihood of disease progression would be higher when failure to BCG treatment occurs. In **p53**-negative patients, approximately 20% presented disease progression.

By and large, we observe that **p53** expression is directly related to prognosis of patients with bladder cancer (TCC), and those with superficial disease and increased **p53** expression must be carefully followed up. Patients with carcinoma in situ treated with BCG who relapse and have positive **p53** must be considered candidates to radical cystectomy.

Those who underwent radical cystectomy and have positive **p53** should be considered as at higher risk for progression, with a strict control.

It is worth mentioning that the immunohistochemical expression of gene **p53** does not change the importance of traditional prognostic factors of bladder cancer, especially the histological grade of cancer. Additionally, there are conflicting data in the literature when analyzing
the role of gene $p53$ expression as an independent factor in prognosis of bladder carcinomas.

A possible explanation for the conflicting results about this gene would be difficult standardization of different techniques to detect gene $p53$ mutations, since none of them has 100% sensitivity.

Another explanation for the controversies would be the different expression of several antibodies, if compared to antibody pab 1801, which present poorer results in detection of mutations in tissues fixed in formalin. On the other hand, agreement of pab 1801 in fresh specimen and paraffin-treated material is approximately 92 to 96%. However, when comparing three antibodies - CM1, Pab 1801 and D07 - in fresh material and in paraffin, it was observed that the main disagreement factor is the variable expression of protein p53 in the same tumor sample, regardless of the antibody used.

Hence, the relation between the several agents acting in tumor biology should be better understood, and the detection techniques should be improved and standardized to determine the clinical role of gene $p53$ expression\(^{(10,11)}\).

**GENOMIC INSTABILITY**

Genomic instability, particularly in transitional cell carcinoma of the bladder with two types, is detected in six out of 200 cases of TCC. Genomic instability in cancer could be divided into two types: microsatellite instability (MSI) and microsome instability (CIN). MSI is related to defects in mismatch repair (MMR) and CIN is related to abnormalities in chromosome segregation.

Considering bladder cancer, 50% is related to CIN and presents worse prognosis and 9% is characterized as MSI with better prognosis; moreover, bladder cancer associated to MSI is more frequent in young patients\(^{(12-17)}\).

**GSTM1 AND CYP2D6**

Susceptibility to bladder cancer depends on the correlation between genetic factors and the environment. Recent researches aimed at associations of susceptibility to bladder cancer and variance in polymorphism of genes CYP2D6 and GSTM1\(^{(18)}\).

Gene CYP2D6 is related to cytochrome P450 whose substrata include aromatic amines and nitrosamines of tobacco.

GSTM1 is related to enzymes with several functions, including deintoxication of the aromatic cycle of hydrocarbons of cigarettes and aromatic amines.

Some studies carried out in patients with bladder cancer analyzed the occurrence of both genes and showed that the gene GSTM1 is more associated to the occurrence of bladder cancer, especially in those patients with superficial tumors and the association is stronger in smokers.

Gene CYP2D6 did not present the same significance of association.

**Amplification/over-expression of a mitotic gene in bladder cancer**

Genomic instability has been suggested as a cause of malignant transformation and progression to cancer; some studies indicate that several genetic instabilities are associated to different mechanisms leading to human cancer.

The best characterized genomic instability is inactivation of gene-repairing DNA; however, this mode of instability can be verified in a small proportion of tumors.

Gene STK15/BTAK/AuroraA is associated with aneuploidy and transformation when overexpressed in cells; when this occurs there is activation of an oncogen involving the amplification of a centrometer and resulting in miscegenation of chromosomes. This aneuploidy, when added to prognostic clinical factors is associated to bladder tumor.

Although high expression of STK15 seems to be frequent in bladder tumors, approximately 30% of aneuploidy in aggressive bladder tumors does not evidence STK15 with overexpression. This suggests that, in a fraction of bladder tumors, other genes are associated to aneuploidy. In fact, two discoveries of members of the Aurora kinase family, Aurora-B and Aurora-C, were reported as responsible for the increased expression of some human cancers.

It is important to note that STK15 is located in chromosome 20q13 and it was identified by the search of sequences with over-expression in chromosome 20. The 20q region is typically amplified in high grade bladder cancer.

Therefore, while analyzing the occurrence of genomic instability, especially in regard to aneuploidy and the occurrence of bladder tumors, it is observed that they are strongly related.

Tumors with a minimum deviation of chromosome copy are clinically less aggressive than those with more deviation of chromosome copies.

Recent investigations showed that STK15/BTAK/AuroraA is involved in the regulation of centrometer and it is linked to frequent amplification and overexpression of human tumors and, therefore, it would be a regulating component of chromosome segregation, and it can cause aneuploidy and transformation.

Hence, it is confirmed that STK15 is associated to increased expression of mitoses and aneuploidy,
and consequently more aggressive feature of bladder cancer\textsuperscript{19}.

**Loss of chromosome 3 allele, microsatellite abnormalities and bladder cancer**

Studies indicated that deletions in chromosome 3, especially three discreet ones in regions 3p are identified in bladder cancer, suggesting that the gene suppressor is in these regions. Detection of the loss of alleles in these regions would also be associated to higher severity of bladder tumor, suggesting its relation with cancer progression.

Other studies indicated that allele loss could occur in chromosomes 17p13, 3p25-26, 9q12-33. Higher occurrence of allele deletion is in chromosome 9p and 9q; molecular maps showed several suppressing genes in chromosome 9 and higher likelihood of loss of chromosome 9 would be an early event in onset of cancer. The other genetic abnormalities that frequently occur are located in chromosomes 1, 8, 16, 14 and 21. Thus, loss of alleles in multiple sites of the genome frequently occurs in bladder cancer.

Upon isolate analysis of carcinoma *in situ* patients, it was observed that this type of tumor contains cells with non-specific loss of chromosome 9; however, neoplasia *in situ* with synchronous carcinoma showed lesions with loss in chromosome 9 and higher likelihood of loss of chromosome 9 would be an early event in onset of cancer. The other genetic abnormalities that frequently occur are located in chromosomes 1, 8, 16, 14 and 21. Thus, loss of alleles in multiple sites of the genome frequently occurs in bladder cancer.

It should be kept in mind that molecular biology and genetics are in the path to new knowledge about tumor molecular pathophysiology and that gene therapy stopped being fiction to be transformed in a real hope.

**CONCLUSION**

With the emergence of new molecular biology techniques, cell abnormalities occurred in carcinogenesis could be studied in more details. Nowadays, as a general concept, it is accepted that the carcinogenic process results from a complex path involving the combination of inactivation of tumor-inhibiting genes and activation of tumor-promoting genes (oncogenes). Several stages must be reached in the formation of a tumor, starting with tumor inhibition, then proliferation, loss of inhibition by contact, invasion and metastasis of the cancer cell.

Accumulation of mutations in essential genes can transform a normal cell into a cancer cell. This transformed cell then grows in a disorganized manner until it forms the tumor with additional mutations occurring during this process. When cancer is diagnosed, it is a mix of cancer cells with subclones, making it difficult to establish the order of occurrence of genetic abnormalities.

Bladder cancer, however, allows a different and unique study of mutations because the different tumor subclones grow in separate sites and allow independent studies.

Several studies were and are being carried out in an attempt to relate the biological characteristics of bladder cancer, its occurrence, histology and its severity with the different types of mutations and chromosome instability.

It should be kept in mind that molecular biology and genetics are in the path to new knowledge about tumor molecular pathophysiology and that gene therapy stopped being fiction to be transformed in a real hope.

**REFERENCES**


